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Citation for final published version:

Rousselot, Philippe, Coude, Marie Magdelaine, Gokbuget, Nicola, Passerini, Carlo Gambacorti, Hayette, Sandrine, Cayuela, Jean-Michel, Huguet, Francoise, Leguay, Thibaut, Chevallier, Patrice, Salanoubat, Celia, Bonmati, Caroline, Alexis, Magda, Hunault, Mathilde, Glaisner, Sylvie, Agape, Philippe, Berthou, Christian, Jourdan, Eric, Fernandes, Jose, Sutton, Laurent, Banos, Anne, Reman, Oumedaly, Lioure, Bruno, Thomas, Xavier, Ifrah, Norbert, Lafage-Pochitaloff, Marina, Bornand, Anne, Morisset, Laure, Robin, Valerie, Pfeifer, Heike, Delannoy, Andre, Ribera, Josep, Bassan, Renato, Delord, Marc, Hoelzer, Dieter, Dombret, Herve and Ottmann, Oliver ORCID: <https://orcid.org/0000-0001-9559-1330> 2016. Dasatinib and low-intensity chemotherapy in elderly patients with Philadelphia chromosome-positive ALL. Blood 128 (6) , pp. 774-782. 10.1182/blood-2016-02-700153 file

Publishers page: <http://dx.doi.org/10.1182/blood-2016-02-700153>  
<<http://dx.doi.org/10.1182/blood-2016-02-700153>>

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**Dasatinib and low-intensity chemotherapy in elderly patients with Philadelphia chromosome-positive ALL.**

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**Running head:** Dasatinib and low chemotherapy in older Ph+ ALL

**Word count:** Text: 3969 (max 4000); Abstract 249 (max 250); Fig/tables: 7 (max 7); Refs: 21.

**Scientific category:** CLINICAL TRIALS AND OBSERVATIONS

## KEY POINTS

- Dasatinib, a potent TKI, combined with low-intensity chemotherapy gave 36% 5-year OS in Ph+ ALL patients over the age of 55 years.
- A low co-morbidity score is prognostic for improved survival in this population.
- Prospective monitoring of mutations may be useful to personalize therapy in Ph+ ALL patients not eligible for intensive therapy.

## ABSTRACT

Prognosis of Philadelphia-positive acute lymphoblastic leukemia (ALL) in the elderly improved during the imatinib era. We investigated dasatinib, another potent tyrosine kinase inhibitor, in combination with low-intensity chemotherapy. Patients older than 55 years were treated with dasatinib 140 mg/day (100 mg/day over 70 years) with intrathecal chemotherapy, vincristine and dexamethasone during induction. Patients in complete remission continued consolidation with dasatinib and reduced doses of sequential cytarabine and methotrexate for 6 months. Maintenance therapy was dasatinib and vincristine dexamethasone re-inductions for 18 months then dasatinib. Seventy-one patients with a median age of 69 years were enrolled, 77% had a high comorbidity score. Complete remission rate was 96% and 65% of patients achieved a 3log reduction in BCR-ABL transcript levels (MMR) during consolidation. Seven patients (10%) underwent allogeneic hematopoietic stem cell transplantation. At 5-years, overall survival was 36% and up to 45% taking into account deaths unrelated to disease or treatment as competitors. In multivariate analysis, a low CIRS-G score and MMR during consolidation were associated with longer overall survival. Thirty-six patients relapsed, 24 were tested for mutation by Sanger sequencing and 75% were T315I-positive. BCR-ABL<sup>T315I</sup> was tested by ASO RT-QPCR in 43 patients and detection was associated with short-term relapses. Ten patients (23%) were positive before any therapy and eight relapsed, all with this mutation. In conclusion, dasatinib combined with low-intensity chemotherapy was well tolerated and gave long-term survival in 36% of elderly patients with Ph+ ALL. Monitoring of BCR-ABL<sup>T315I</sup> from diagnosis may help to switch therapy before hematologic relapse.

## INTRODUCTION

Philadelphia chromosome is the most frequent recurrent cytogenetic abnormality in elderly acute lymphoblastic leukemia (ALL) patients. Its incidence increases with age, reaching approximately 50% in ALL patients aged 60 years and over.<sup>1</sup> Until the recent era of tyrosine kinase inhibitors (TKIs), most studies devoted to elderly patients with ALL made no distinction between Philadelphia chromosome-positive (Ph+) and Philadelphia chromosome-negative cases. This was largely due to equally poor survival irrespective of Philadelphia chromosome status and the absence of differential treatments based on the identification of Ph+ ALL.<sup>2</sup> The Ph chromosome arises from a reciprocal translocation between chromosomes 9 and 22 (t(9;22)(q34;q11.2)) fusing the c-ABL tyrosine kinase (TK) gene on chromosome 9 and the breakpoint cluster region (BCR) gene on chromosome 22. The deregulated BCR-ABL tyrosine kinase activity plays a major role in a transformation process in conjunction with other genetic alterations leading to a differentiation block giving rise to Ph+ (or BCR-ABL1 positive) ALL.<sup>3</sup>

Designed as a targeted therapy against BCR-ABL tyrosine kinase activity, imatinib rapidly became a gold standard therapy in chronic myeloid leukemia and was proposed in relapsed/refractory Ph+ ALL.<sup>4</sup> The addition of imatinib to age-adapted chemotherapy significantly improved complete remission (CR) rates and disease-free survival as compared to historical controls in elderly Ph+ ALL patients.<sup>5</sup> A randomized study from the German Multicenter Study Group for Adult ALL (GMALL) established the benefit of imatinib against a chemotherapy-based induction.<sup>6</sup> To reduce toxicity, the Italian Group for Haematological Diseases in Adults (GIMEMA) proposed combining high-dose imatinib (800 mg/day) with prednisone, without chemotherapy.<sup>7</sup> Preliminary data from the combination of high-dose imatinib with vincristine and dexamethasone (DIV regimen) suggested that a low-intensity chemotherapy approach was feasible and effective both in relapsed and in elderly Ph+ ALL patients.<sup>8</sup>

The impact of new tyrosine kinase inhibitors, such as dasatinib or nilotinib, on the prognosis of Ph+ ALL is being prospectively assessed.<sup>9</sup> Dasatinib is an oral, multi-targeted kinase inhibitor of the BCR-ABL and SRC family kinases, originally designed as an SRC kinase inhibitor. Dasatinib can bind to both the active and inactive conformations of the ABL kinase domain. Since it has less stringent binding requirements than imatinib, dasatinib has activity against many imatinib-resistant kinase domain mutations of BCR-ABL. One exception is the T315I mutation within the ATP-binding pocket of the ABL tyrosine kinase that confers a high degree of resistance.<sup>10</sup>

The European Working Group on Adult ALL (EWALL) presented a consensus defining a low-intensity chemotherapy backbone for adult ALL patients aged 55 years and over, the “EWALL backbone”. In the present study, we combined the EWALL backbone with dasatinib (Sprycel®, Bristol-Myers Squibb), for Ph+ ALL patients aged 55 years and over.

## **PATIENTS AND METHODS**

### **Study design and population**

EWALL-PH-01 is the first prospective phase 2 study conducted by the EWALL group on behalf of the European Leukemia Net. Patients aged 55 years or older were eligible if they had newly diagnosed Ph-positive and/or BCR-ABL-positive ALL with or without documented central nervous system (CNS) involvement and if they had not been previously treated, except with corticosteroids or single dose vincristine. Patients had to have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 3. Ineligibility criteria were QTc > 470 ms, heart insufficiency NYHA grade III/IV, left ventricular function <50%, myocardial infarction within 6 months prior to the study, active secondary malignancy or inadequate hepatic functioning defined as ASAT or ALAT > 2.5 times the institutional upper limit of normal (ULN) and total bilirubin > 2 x ULN unless due to organ involvement. Written informed consent was obtained from all patients. The study was approved in May 2007 by the institutional review board of Ile de France XI, France. The study was conducted in accordance with the Declaration of Helsinki (EudraCT: 2006-005694-21) and is registered on the EU Clinical Trial Web site (<https://www.clinicaltrialsregister.eu/ctr-search/trial/2006-005694-21/FR>).

### **Study treatments**

The planned protocol is summarized in Figure 1. After a pre-phase, dasatinib was administered at 140 mg QD during the induction period in combination with weekly vincristine 2 mg intravenous (IV) (1 mg for patients over 70 years) and dexamethasone 40 mg for 2 days (20 mg for patients over 70 years) for 4 weeks. Consolidation consisted of dasatinib 100 mg/day sequentially with methotrexate 1000 mg/m<sup>2</sup> IV day 1 and asparaginase 10000 UI/m<sup>2</sup> IM day 2 for cycles 1, 3 and 5 and cytarabine 1000 mg/m<sup>2</sup>/12 h IV day 1, day 3, day 5 for cycles 2, 4 and 6, with 4-week cycles. Maintenance consisted of dasatinib 100 mg/day sequentially with 6-mercaptopurine (60 mg/m<sup>2</sup>/day) and methotrexate (25 mg/m<sup>2</sup>/week) orally, one every other month and dexamethasone/vincristine every 2 to 3 months up to 24 months. Post-maintenance therapy consisted of dasatinib alone (100 mg/m<sup>2</sup>/day) until relapse or death. The protocol allowed hematopoietic stem cell transplantation (HSCT) with reduced-intensity conditioning (RIC) or myeloablative conditioning (MAC) in eligible patients. CNS prophylaxis included alternating intrathecal therapy with methotrexate (15 mg), cytarabine (40 mg) and prednisone (40 mg) for a total of six injections (four during induction and two during consolidations). Due to an excess rate of treatment discontinuation in the first 11 patients over 70 years receiving dasatinib 140 mg/day, the protocol was amended after 15 months to reduce dasatinib and chemotherapy doses for patients aged 70 years and older (dasatinib 100 mg/day during induction, methotrexate 500 mg/m<sup>2</sup>, asparaginase 5000 UI/m<sup>2</sup> and cytarabine 500 mg/m<sup>2</sup> during consolidations). Comorbidities were assessed at inclusion by the Cumulative Illness Rating Scale for Geriatrics comorbidity index (CIRS-G).<sup>11</sup>

### **Molecular and cytogenetic assessments**

Patients were monitored for BCR-ABL by RTQ-PCR in national central laboratories in each country in accordance with current recommendations.<sup>12</sup> Minimal residual disease (MRD) was assessed after induction (MRD1), during consolidation (MRD2) and throughout maintenance. Bone marrow (BM) results were considered preferentially when results from both peripheral blood (PB) and BM were available. In the absence of a BM sample, PB results were analyzed if available. During consolidation, the highest BCR-ABL value was considered to define MRD2.

Methods for BCR-ABL<sup>T315I</sup> ASO RTQ-PCR are described in the supplemental appendix. A sensitivity threshold of 0.05% was chosen, corresponding to the upper limit of the 95% confidence interval (CI) estimated from the distribution of non-specific signals measured in 60 samples that were negative for BCR-ABL<sup>T315I</sup>. Cytogenetic analyses were performed in each local laboratory at diagnosis and were reviewed centrally according to the classification proposed by Heerema et al.<sup>13</sup>

## **Response definitions**

Complete hematologic remission (CR) was defined as the presence of 5% or less blasts in the bone marrow, with a granulocyte count  $\geq 1.0 \times 10^9/\text{L}$ , platelet count  $\geq 100 \times 10^9/\text{L}$  and no extramedullary disease. Molecular response 5 log (MR5) was defined by a 5 log reduction in BCR-ABL transcript level. Major molecular response (MMR) was defined by a BCR-ABL/ABL ratio  $<0.1\%$ . Relapse was defined by recurrence of more than 5% lymphoblasts in the bone marrow aspirate or by the presence of extramedullary disease after achieving CR. Induction death was defined as death occurring after start of therapy without meeting the definition of CR or resistant disease. Resistant disease included patients who survived the induction treatment period but had persistent leukemia. Relapse-free survival (RFS) was calculated from the first day of therapy to the relapse date. Event-free survival (EFS) was defined as the time between randomization and primary refractoriness, hematologic relapse or death in first CR, other patients being censored at the time of last contact. Overall survival (OS) was calculated from the date of initiation of therapy until death. Toxicity was evaluated according to the National Cancer Institute Common Toxicity Criteria Version 2.0.55.

## **Statistical methods**

The primary endpoint was RFS at 12 months. Patients in remission were censored at the time of last follow-up. A sample size of at least 50 patients was calculated by Fleming's single-stage design to ensure 80% power for proving lack of efficacy if the true CR rate was below 60%. Considering a rate below 40% would imply lack of efficacy, a sample size of at least 48 patients had to be recruited to detect a remission rate  $> 60\%$  with 80% power and a level of  $\alpha=2.5\%$  (one-sided test). Allowing for potential censored data, a total of 55 patients were planned. This number was subsequently increased to 71 patients by amendment to compensate for early treatment discontinuation seen in patients aged over 70 years. Secondary endpoints were cumulative incidence of relapse (CIR), cumulative incidence of non-relapse related mortality, and OS. The Kaplan-Meier method was used to estimate the EFS, RFS and OS probabilities. Hazard ratios (HRs) were given with the 95% confidence interval (CI). Analysis followed the intent-to-treat (ITT) principle. Baseline characteristics were compared by non-parametric tests, either the exact Fisher's test for qualitative variables and the Kruskal-Wallis test for quantitative variables. The censored endpoints (time to death, RFS) were estimated by the non-parametric Kaplan-Meier method with a 95% CI. Cumulative incidences were estimated using Aalen estimator.<sup>14</sup> Prognostic factors of survival and progression-free survival (PFS) were investigated using Cox proportional hazard models. Statistical analysis was performed with XLSTAT software (version 2014.2.03, Addinsoft) and R Project for Statistical Computing software (R version 2.15.2). M.D. and Ph.R analyzed the data and all authors had access to primary clinical trial data.

## **RESULTS**

### **Patient characteristics**



Seventy-one patients were included between August 2007 and July 2010. One patient had CNS involvement at diagnosis (Table 1). Median age was 69 years (range, 59 to 83) and the sex ratio (M/F) was 0.73. Median follow-up on study was 32 months (range, 2 to 88 months) and median follow-up for surviving patient was 66 months (range, 21 to 88 months) at the time of analysis in January 2015. Median CIRS-G score value was 6 (range, 0 to 16). Overall, 77% of the patients had a CIRS-G score >4. Karyotype analysis was performed in all patients and cytogenetic results were evaluable in 63 (90%). All evaluable patients had the t(9;22) chromosomal translocation and 48 (76%) presented with additional cytogenetic aberrations (ACAs). BCR-ABL RQ-PCR was available in all patients. Minor breakpoint transcripts e1a2 and e1a3 encoding for the p190<sup>BCR-ABL</sup> protein were identified in 54 patients (76%). Major breakpoint transcripts e13a2 and e14a2 encoding the p210<sup>BCR-ABL</sup> protein were identified in 17 patients (24%).

### **Response to therapy and outcome**

Sixty-seven of the 71 patients (96%) achieved CR. Four patients failed to obtain CR, one was refractory and obtained CR after salvage therapy and three died during induction. Among the 67 patients in CR after induction, 36 relapsed (54%). Ten patients obtained a second CR (28%), six of whom relapsed again. Forty-nine patients died (69% of the total population) including 32 deaths in relapse or refractory disease and 14 deaths in CR (non-relapse related mortality 29%). Treatment-related mortality was reported in six patients (12%). Eight deaths were unrelated to disease or therapy (including three late deaths, two from adenocarcinoma after 49 and 66 months in study and one from meningioma after 68 months). Twenty-two patients (31% of the total population) were alive at the time of analysis. Seven patients underwent allogenic HSCT, five with RIC and two with MAC. Three patients relapsed after transplant at 4.6, 6.3 and 6.5 months and died, four patients were alive at 53, 56, 59 and 65 months. Figure 2 summarizes treatment outcomes.

Among the 67 patients in first CR, 55 (82%) were evaluable for MRD1. Characteristics of these 55 patients did not differ from those of the 12 CR patients not tested. Thirty-three patients reached MMR (60%) and 11 patients reached MR5 (20%). At MRD2, 36 patients reached MMR (65%) and 13 patients reached MR5 (24%).

RFS at 12 months was 58% [95% CI, 45-69]. At 3 years, RFS, EFS and OS were 33% [95% CI, 22-44], 31% [95% CI, 21-42] and 41% [95% CI, 29-52] respectively. At 5 years RFS, EFS and OS were 28% [95% CI, 18-39], 27% [95% CI, 17-37] and 36% [95% CI, 25-47] respectively. Median RFS, EFS and OS were 19.1, 18.9 and 25.8 months respectively. Taking into account deaths unrelated to disease or treatment as competitors, OS was 45% [95% CI, 43- 67] at 5 years (Figure 3B). Taking allogenic HSCT as competing events did not impact RFS, EFS or OS (Supplementary Figure 1).

### **Safety**

Median time on dasatinib therapy was 7.8 months (range 0.6-72.4). The cumulative incidence of dasatinib discontinuation for any reason is illustrated in Supplementary Figure 3. Twelve out of 22 patients alive were still on dasatinib at the time of analysis (54%). The other ten patients were being treated with imatinib or nilotinib.

During induction, the mean duration of neutropenia < 0.5 G/L was 8.9 days (range, 0 to 41 days) and mean duration of fever > 38.5°C was 3.3 days (range, 0 to 10 days). G-CSF was administered to 73% of

the patients and 23 patients (32%) did not experience neutropenia. Mean duration of large spectrum antibiotics was 12 days. Thirteen patients presented bacteremia or septicemia (four with gram-negative and nine with gram-positive strands). Two patients developed nonbacterial lung infections (one pneumonia with *Pneumocystis jirovecii* and one *Aspergillosis*). Mean duration of platelets <20 G/L was 3 days (range, 0 to 41 days). Consolidations with methotrexate and asparaginase were associated with very few episodes of neutropenia (two during consolidation 1, two during consolidation 3, and none during consolidation 5). Consolidation with intermediate-dose cytarabine was associated with a mean duration of neutropenia < 0.5 G/L of 4.2 days, 5.9 days and 6.3 days during consolidation 2, 4 and 6, respectively, and a mean duration of platelets < 20 G/L of 3.2 days, 4.5 days and 4.9 days, respectively. During maintenance, 11 patients presented short episodes of neutropenia < 0.5 G/L.

Pleural effusion occurred in seven patients (10%), including three cases during induction, two cases during consolidation, one case during maintenance and one case during post-maintenance therapy with dasatinib. The median age of patients with pleural effusion was the same as that of patients without. No concomitant lymphocytosis was seen. Median latency was 2.3 months with only one late case (after 60.7 months). Three patients were receiving 140 mg/day and four 100 mg/day at the onset of pleural effusion was 100 mg/day. Median duration of pleural effusion was 13 days (range, 4 to 41 days). As has previously been reported elsewhere,<sup>15</sup> four cases were associated with interstitial pneumonia and one case with pericardial effusion. In the late-occurring case there was a strong suspicion of pulmonary hypertension on an echocardiography in this patient also diagnosed with peritoneal carcinomatosis (Supplementary Table 2). Of note, RFS of patients who experienced pleural effusion was not different to those without (Supplementary Figure 2).

Other serious adverse events included tumor lysis syndrome during induction (n=2), renal failure (n=6), subdural hemorrhage (n=3, including one patient with concomitant acetyl salicylic acid therapy), digestive hemorrhage (n=3), transaminase elevations (n=5), pulmonary embolism (n=3), atrial fibrillation (n=4), and cardiac failure (n=2).

### Relapses and mutations

Thirty-six relapses were observed including 14 during consolidation, 19 during maintenance and 3 after allogeneic HSCT. Cumulative incidence of relapse was 54% [95% CI, 42-66] at 5 years. Standard mutation analysis with Sanger sequencing was available at relapse for 24 patients: 18 relapses were associated with the T315I mutation (including one compound mutation T315I+M244V+E255K), one relapse was associated with the F317L mutation, one with the V299L, one with a compound mutation without T315I, and mutations were not detectable in three patients. Sanger sequencing was not performed in 12 patients at relapse but ASO PCR for the detection of BCR-ABL<sup>T315I</sup> was negative in all seven patients tested (Figure 4).

ASO PCR for the detection of BCR-ABL<sup>T315I</sup> was retrospectively performed in 43 patients on prospectively-collected RNA samples. No significant differences were seen between tested and non-tested patients in terms of baseline characteristics. Of these 43 patients, 10 (23%) were BCR-ABL<sup>T315I</sup>-positive by ASO PCR at the sensitivity level of 0.05% before any therapy (Figure 5). No mutation was detectable by Sanger sequencing on these samples. Eight patients relapsed, all with the T315I mutation including one relapse 4 months after allogeneic HSCT. During follow-up, BCR-ABL<sup>T315I</sup> was detected 1 to 3 months before hematologic relapse. Two patients did not relapse, one patient was transplanted and was still in CR 54 months after allogeneic HSCT, and the other died in CR at 9.6 months from lung

adenocarcinoma. We compared the cumulative incidence of relapse (death was considered as a competing risk) between T315I mutated (BCR-ABL<sup>T315I</sup>) and other patients at diagnosis. Quicker relapses were observed in cases of a detectable BCR-ABL<sup>T315I</sup> (median 7 months) as compared to other patients (median not reached) (Fine and Gray regression coefficient: 14.80 [95%CI, [7.03-31.20]],  $p<0.001$  (Figure 6).

### Prognostic factors

Univariate and multivariate analyses were performed including age and CIRS-G score (continuous variables), sex, leukocytes  $<30 \times 10^9/L$ , karyotype, BCR breakpoint, BCR-ABL<sup>T315I</sup> at diagnosis, and molecular response status at MRD1 and MRD2. In univariate analysis, younger age (HR, 0.95 [95% CI, 0.90-0.99];  $p=0.048$ ), female gender (HR, 0.46 [95% CI, 0.23-0.77];  $p=0.0058$ ), leukocytes  $<30 \times 10^9/L$  (HR, 0.52 [95% CI, 0.26-0.90];  $p=0.002$ ), and no BCR-ABL<sup>T315I</sup> at diagnosis (HR, 0.43 [95% CI, 0.12-0.83];  $p=0.002$ ) were associated with longer OS. Better RFS was associated with leukocytes  $<30 \times 10^9/L$  (HR, 0.56 [95% CI, 0.28-0.97];  $p=0.04$ ), no ACAs (HR, 0.45 [95% CI, 0.27-0.95];  $p=0.03$ ), no BCR-ABL<sup>T315I</sup> at diagnosis (HR, 0.43 [95% CI, 0.11-0.91];  $p=0.03$ ) and achievement of MMR at MRD2 (HR, 0.45 [95% CI, 0.15-0.85];  $p=0.02$ ).

Detection of BCR-ABL<sup>T315I</sup> at diagnosis was excluded from the multivariate analysis given that all patients were not tested. For OS, female gender (HR, 0.28 [95% CI, 0.13-0.63];  $p=0.002$ ), a low CIRS-G score (HR, 0.79 [95% CI, 0.69-0.91];  $p=0.001$ ), and achievement of MMR at MRD2 (HR, 0.31 [95% CI, 0.12-0.78];  $p=0.013$ ) were associated with increased OS. Similarly, a low CIRS-G score (HR, 0.76 [95% CI, 0.65-0.90];  $p=0.001$ ) and achievement of MMR at MRD2 (HR, 0.17 [95% CI, 0.06-0.51];  $p=0.002$ ) were associated with longer RFS. A trend was observed for longer RFS in patients without ACAs ( $p=0.05$ ).

## DISCUSSION

This study reports the long term outcome of elderly Ph+ ALL patients treated with dasatinib, a second generation TKI in combination with low-intensity chemotherapy. Complete remission was achieved in 96% of the patients, RFS at 12 months was 58% and 5-year overall survival 36%.

Relatively few studies have specifically investigated the use of imatinib in combination with chemotherapy in Ph+ ALL in the elderly. The GIMEMA proposed a study in Ph+ ALL patients aged more than 60 years evaluating the use of steroids combined with high-dose imatinib (800 mg/day), without additional chemotherapy. Complete remission was obtained in all 29 patients with low toxicity however remission duration was limited in patients not eligible for allogeneic HSCT.<sup>7</sup> The GMALL group performed a randomized study assessing induction therapy with either imatinib or multi-agent, age-adapted chemotherapy followed by imatinib administered with consolidation chemotherapy in both arms. The overall CR rate was 96% in patients assigned to imatinib and 50% in patients allocated to chemotherapy. OS was not significantly different between the two cohorts.<sup>6</sup> A low-intensity schedule (vincristine and dexamethasone) in combination with high-dose imatinib (800 mg/day) was proposed in the GRAALL AFR07 study for patients aged more than 55 years (DIV regimen).<sup>8</sup> A pilot study of this combination showed promising results in relapsing and refractory Ph+ ALL with a CR rate of 90% in patients older than 55 years suggesting that high-dose chemotherapy may not be needed in this population.

The question as to whether dasatinib improves the prognosis of Ph+ ALL patients has been investigated in a few studies. The GIMEMA group tested an induction regimen combining dasatinib at the initial dose of 70 mg twice daily with intrathecal chemotherapy and steroids for 84 days.<sup>16</sup> All 53 patients (median age 54 years) achieved CR, and disease-free survival was 51% at 20 months despite intensive consolidation therapies in 60% of the patients. A second study tested the combination of intermittent dasatinib (100 to 140 mg/day) with the hyper-CVAD regimen in 72 patients (median age 55 years). Response rate was 96% and long term survival was 46% with a median follow-up of 67 months for surviving patients.<sup>17</sup> The median age of the patients included in the current EWALL-PH-01 study (69 years) is the oldest reported to date in a large cohort. This elderly group of patients had a very low rate of allogeneic HSCT (10%). In this setting, the combination of dasatinib with low-intensity chemotherapy gave 5-year OS of 36%, suggesting that a subgroup of elderly patients may experience long-term survival without the need for intensive therapies.

The achievement of MMR during consolidation (MRD2) was a strong predictor for OS and RFS in this study, which is contrary to other reports that MMR after induction has not shown prognostic value.<sup>18</sup> Sixty-five percent of the patients reached MMR at MRD2, a proportion similar to that observed in the GRAAPH study for young patients using imatinib and intensive consolidation.<sup>19</sup> The deep molecular response rate was also comparable suggesting that molecular response can also be achieved with a more potent TKI without the need for intensive consolidation regimens.

This is the first demonstration of the comorbidity score (CIRS-G) as a prognostic factor for survival in elderly Ph+ ALL. Overall, 77% of the patients included in the EWALL-PH-01 study had a CIRS-G score > 4, highlighting the growing importance of deaths unrelated to the disease in elderly patients. Eight of the patients died from causes independent of ALL or therapy. Five-year OS was 45% taking into account deaths unrelated to disease or treatment as competitors.

Safety was acceptable in this elderly population, especially after the reduction of dasatinib daily dose from 140 to 100 mg/day for patients aged 70 years or more. Despite the use of a more potent TKI, the rate of induction deaths was low (4%). Treatment-related mortality (12%) compares favorably with previous studies using more intensive chemotherapy.<sup>7</sup> Pleural effusions are age and dose-dependent in patients treated with dasatinib. Recent long-term follow-up studies reported an incidence of 28% to 35% at 5 years in CML patients treated with imatinib compared to only 10% in our aged ALL population.<sup>20</sup> This low rate of pleural effusions in our study is likely related to the concomitant administration steroids and immunosuppressive chemotherapy.

Relapses were associated with clones harboring mutations known to confer a high degree of resistance to dasatinib, as previously described.<sup>21</sup> Sanger sequencing was performed in 24 out of 36 relapses and the T315I mutation was present in 18 of 24 relapsing patients (75%). Other mutations which may be selected by dasatinib were identified such as the V299L mutation (one case) and the F317L mutation (one case). With a specific ASO-PCR for the detection of BCR-ABL<sup>T315I</sup>, we were able to search for the T315I mutation at diagnosis in 43 patients. Ten patients (23%) were found to be positive. For the first time, we have been able to assign a negative prognostic value of this finding at the level of sensitivity of 0.05%. Eighty percent of the patients detected with positive BCR-ABL<sup>T315I</sup> ASO-PCR relapsed except two patients, one who died in CR during maintenance and one who underwent allogenic HSCT. Similarly, 80% of the patients detected as BCR-ABL<sup>T315I</sup>-positive during follow-up relapsed one to three months after detection.

Whether the use of more powerful BCR-ABL inhibitors such as ponatinib may improve OS in Ph+ ALL remains to be determined. The cardiovascular toxicity of ponatinib may counteract the potential benefit of this drug in an elderly population with a high comorbidity index, such as that of the EWALL-PH-01 study. A tailored choice based on patient status and adverse disease-related risk factors, such as the monitoring of mutations, could be one way to balance risk and efficacy in future trials.

In conclusion, we report high efficacy of dasatinib combined with low-intensity chemotherapy (EWALL backbone) in elderly Ph+ ALL patients with a 36% long-term survival without the need for intensive chemotherapy or allogenic HSCT. These good-risk patients are defined by a low MRD2 level and a low comorbidity score. We report the first evidence supporting prospective monitoring of the T315I mutation in patients not eligible for intensive therapies in order to personalize therapy before hematologic relapse.

## **Acknowledgments**

We thank the patients and their families for participating in the trial; J Hermann for his help in data monitoring in Germany; For Drug Consulting for pharmacovigilance monitoring and M Hesham for his longstanding support and friendship. This study (#EudraCT: 2006-005694-21) was sponsored by the Versailles Clinical Research Office (DRCI, “Délégation à la Recherche Clinique et à l’Innovation”, Versailles, France) and supported by research grants from Bristol-Myers Squibb. Bristol-Myers Squibb provided dasatinib. Sarah MacKenzie, PhD (medical writer) supported by funding from Hôpital de Versailles, provided editorial assistance.

## **Authorship contributions**

Contribution: Ph.R., O.O., H.D., D.H., N.G., A.D., J.R., R.B designed the study; L.M. provided administrative support; Ph.R., C.G.P., F.H., T.L., C.S., F.W., M.A., M.H., M.J., Ph.A., C.B., E.J., J.F., L.S., A.B., O.R., B.L., X.T., N.I., A.D., J.R., H.D. and O.O. treated patients and collected data; Ph.R. and O.O. analyzed data and wrote the manuscript; A.B. provided geriatric expertise; JM.C., S.H. and H.P. performed the MRD studies; M.M. performed ASO PCR analysis; M.D. and Ph.R performed the statistical analysis.

## **Conflicts of interest**

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## TABLES AND FIGURES

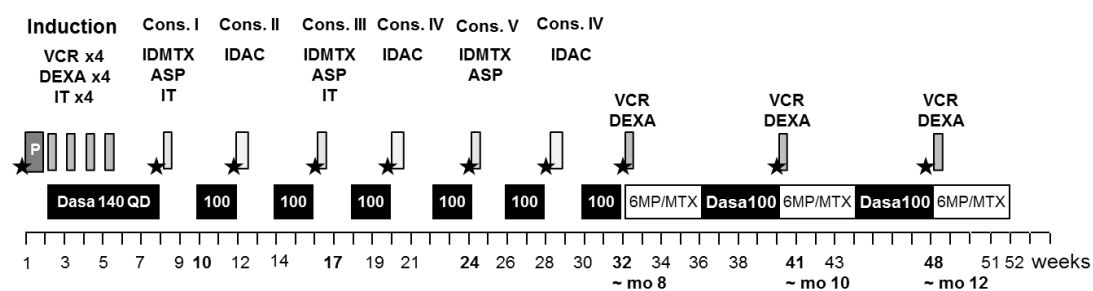
**Table 1. Patient characteristics**

	<b>Patients (n=71)</b>
<b>Baseline</b>	
Male/female, n	30/41
Median age, y (range)	69 (59-83)
ECOG PS 0/1/2/3, n	21/44/5/1
Median CIRS (range)	6 (0-16)
CIRS-G > 4, n (%) <sup>*</sup>	54 (77%)
CNS disease, n (%) <sup>*</sup>	1 (1%)
Median WBC, 10 <sup>9</sup> /L (range)	12.4 (0.8-348.3)
WBC ≥30 x 10 <sup>9</sup> /L, n (%)	27 (38)
<b>Karyotype<sup>**</sup></b>	
Non evaluable, n (%)	8 (11%)
t(9;22) + no secondary aberration, n (%)	15 (21%)
t(9;22) + loss and/or gain, n (%)	31 (44%)
Loss (del(7p), -7, del(9p))	12 (17%)
Gain (+Ph, High Hyperdiploidy)	11 (15%)
Loss and gain	8 (11%)
t(9;22) + others	17 (24%)
<b>Molecular biology, n</b>	
Bcr subtype (m/M/both)	54/17/0

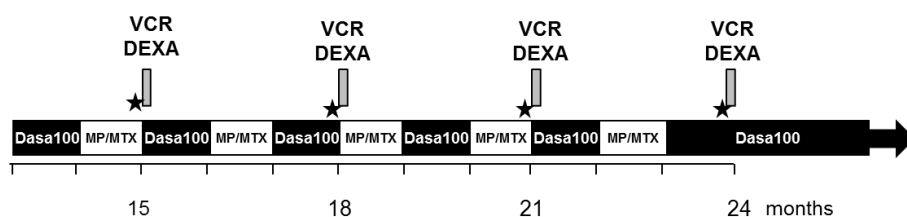
ECOG PS, Eastern Cooperative Group performance status; CIRS, cumulative illness rating scale; WBC, white blood cell count; <sup>\*</sup>n=70; <sup>\*\*</sup> according to Heerema NA et al.<sup>13</sup>; Del(7)p, deletion 7p; -7, monosomy 7; del(9p), deletion 9p; +Ph, duplication of the Ph chromosome; Bcr, BCR-ABL breakpoint; m, minor breakpoint; M, major breakpoint.

**Figure 1. EWALL-PH-01 treatment strategy**

**Induction and Consolidation Therapy (1st year)**

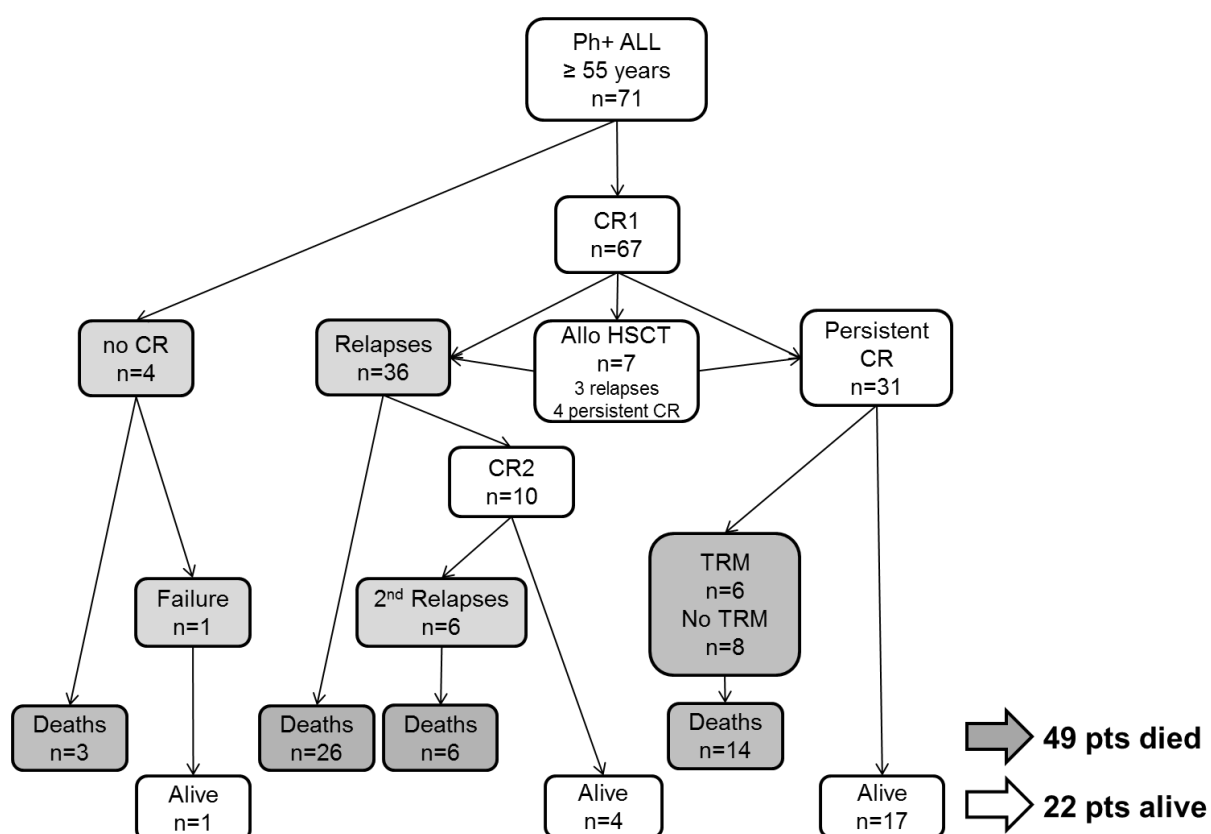


**Maintenance Therapy (2nd year)**



★PCR analysis; ASP, asparaginase; IDAC, intermediate-dose cytarabine; Cons., consolidation; Dasa, dasatinib; DEXA, dexamethasone; IDMTX, intermediate-dose methotrexate; IT, intrathecal (triple IT, 15 mg MTX, 40 mg AraC and 40 mg prednisone); 6MP, 6-mercaptopurine; MTX, methotrexate; mo, month; P, pre-phase; QD, once a day; VCR, vincristine.

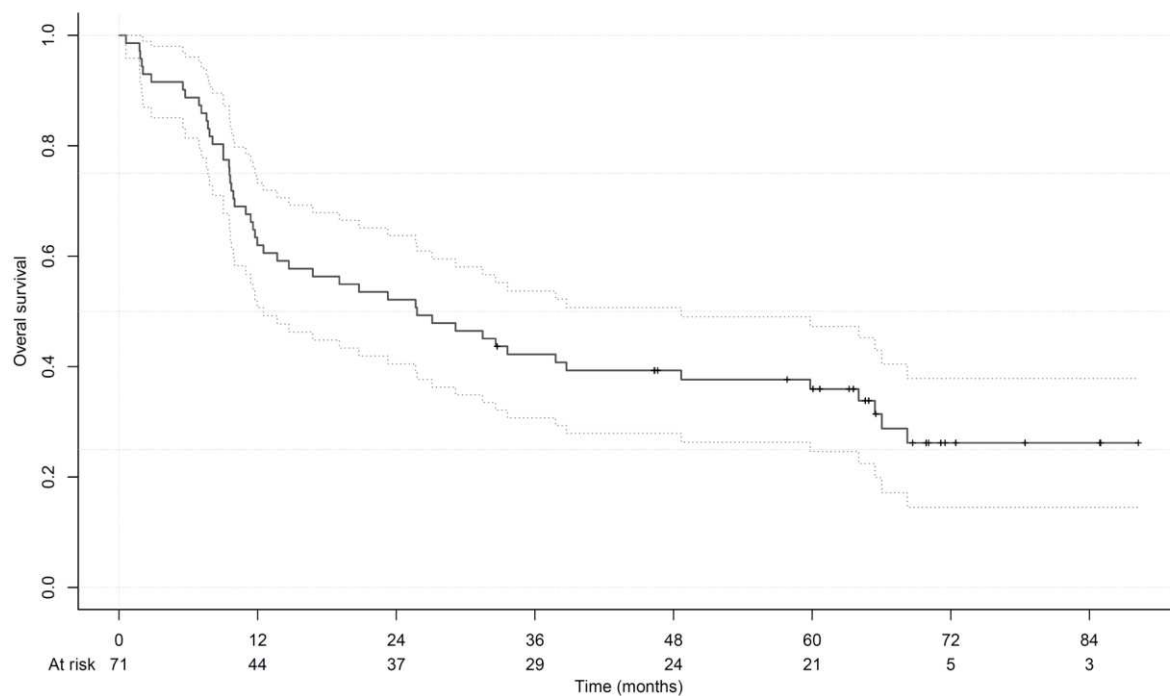
**Figure 2. Consort diagram.**



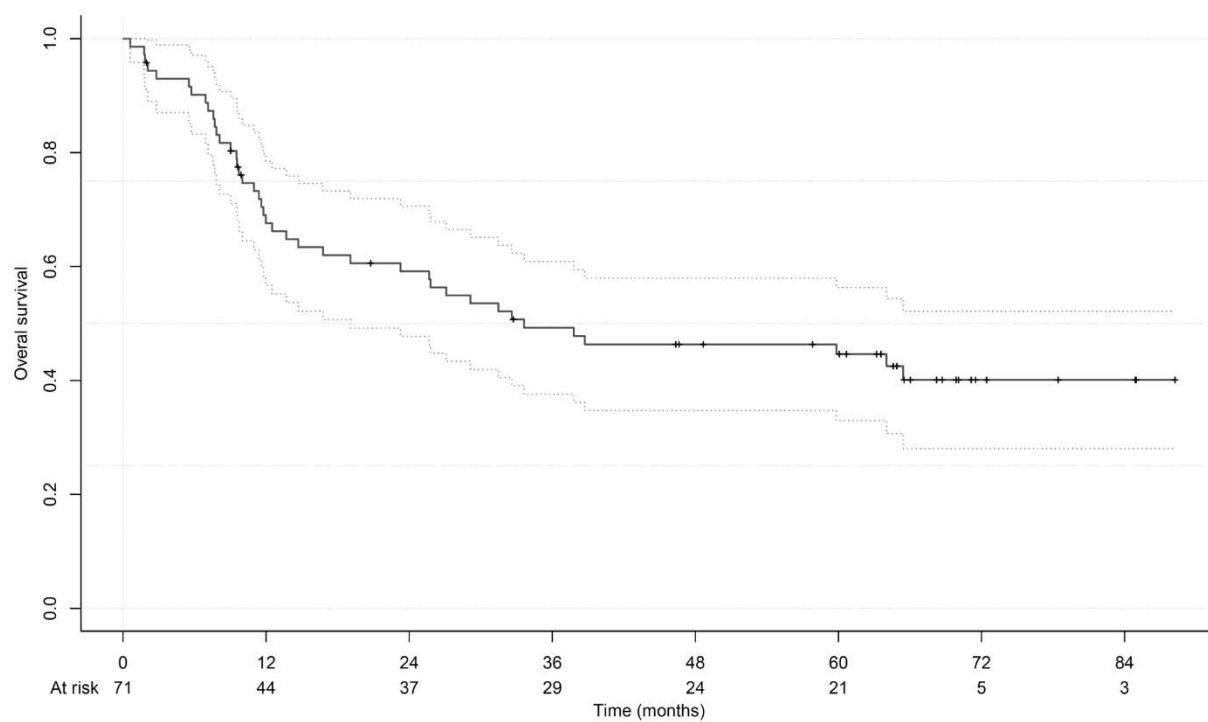
Ph+ ALL, Philadelphia chromosome (or BCR-ABL) positive acute lymphoblastic leukemia; CR, complete remission; CR1, complete remission 1; CR2, complete remission 2; Allo HSCT, allogeneic hematopoietic stem cells transplantations; TRM, treatment related mortality; pts, patients.

**Figure 3. Overall survival (3A, n=71) and overall survival taking into account deaths unrelated to disease or treatment as competitors (3B, n=71), event-free survival (3C, n=71), and relapse-free survival (3D, n=67).**

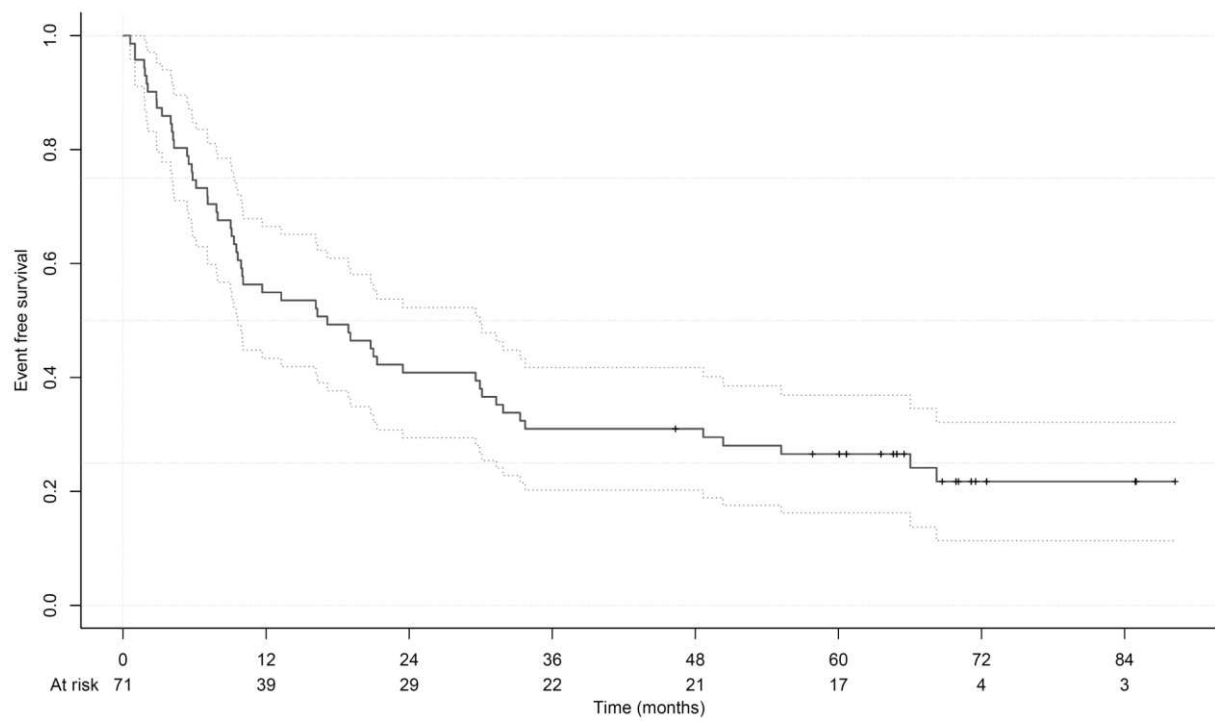
3A.



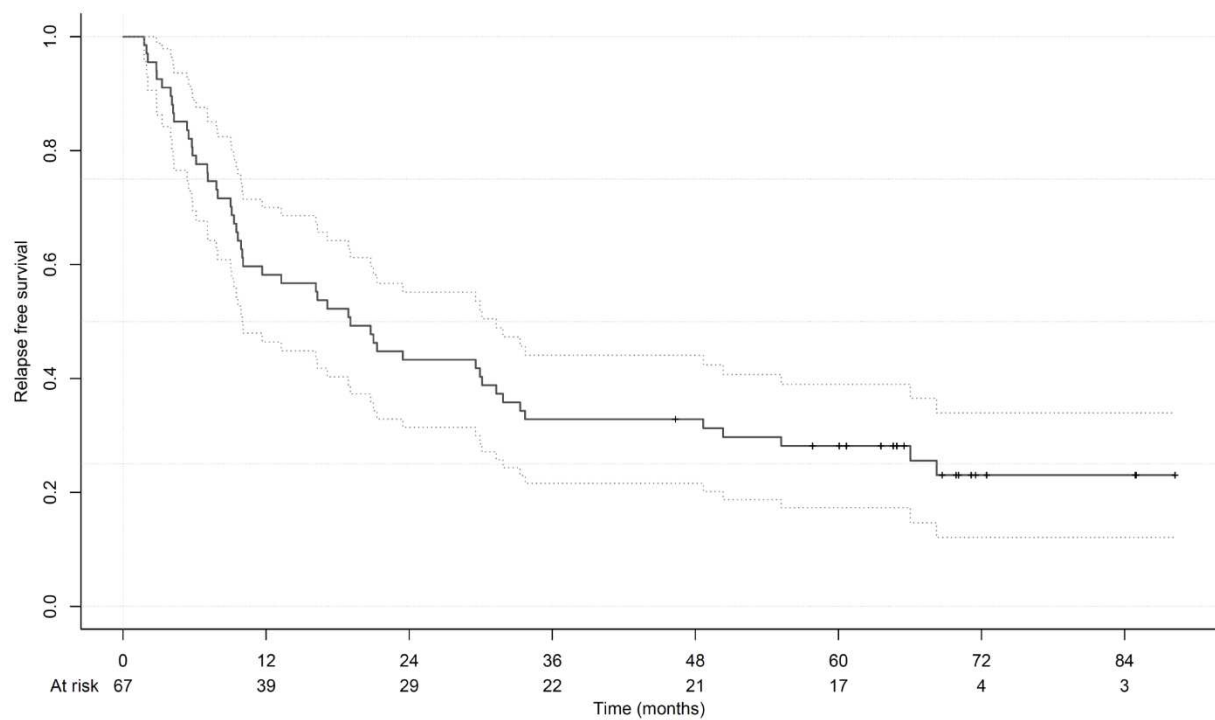
3B.



3C.



3D.



**Figure 4. Distribution of BCR-ABL tyrosine kinase domain mutations in patients in first relapse.**

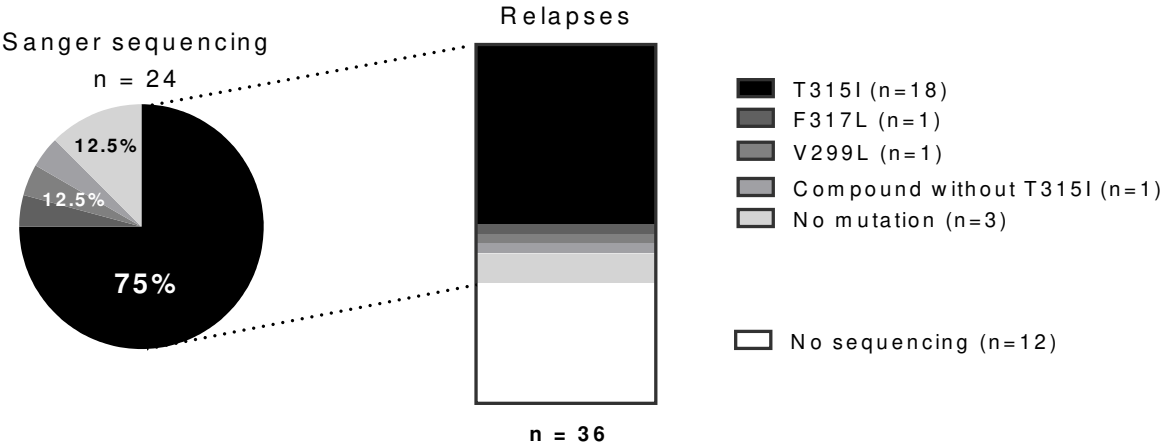
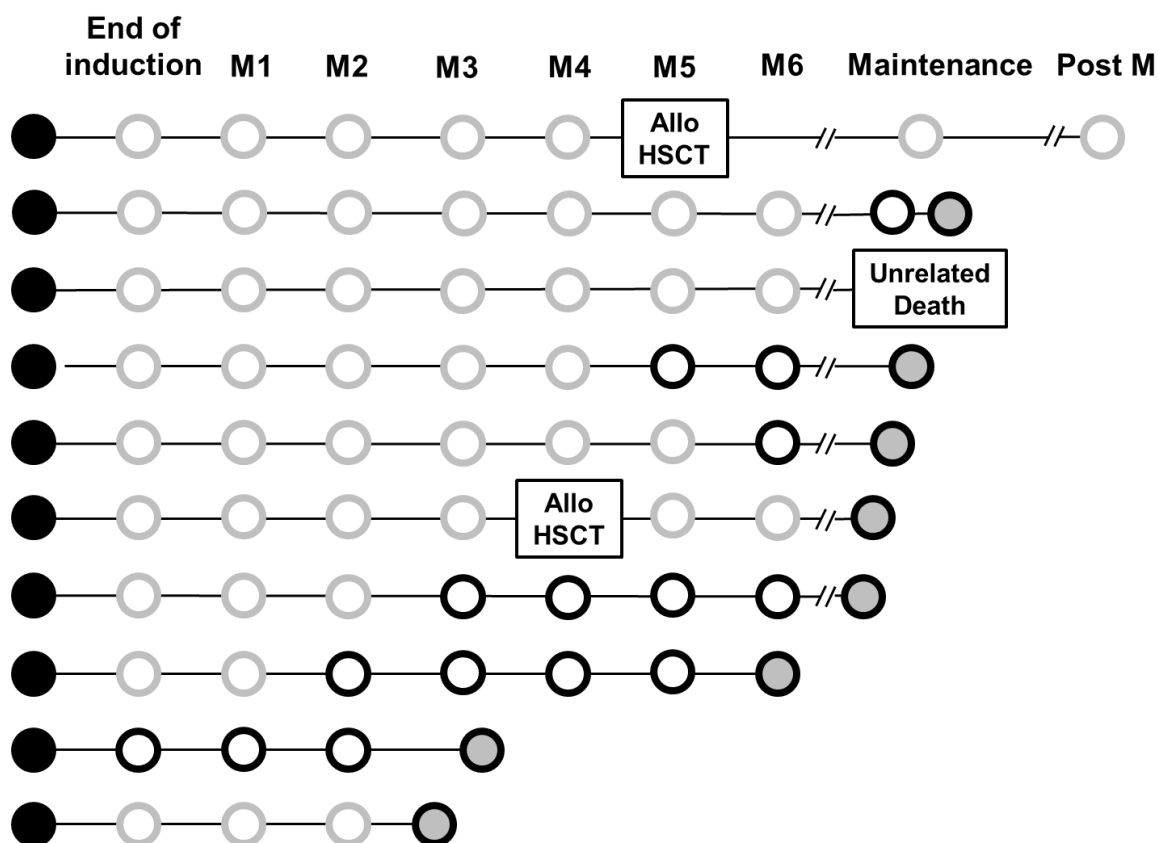


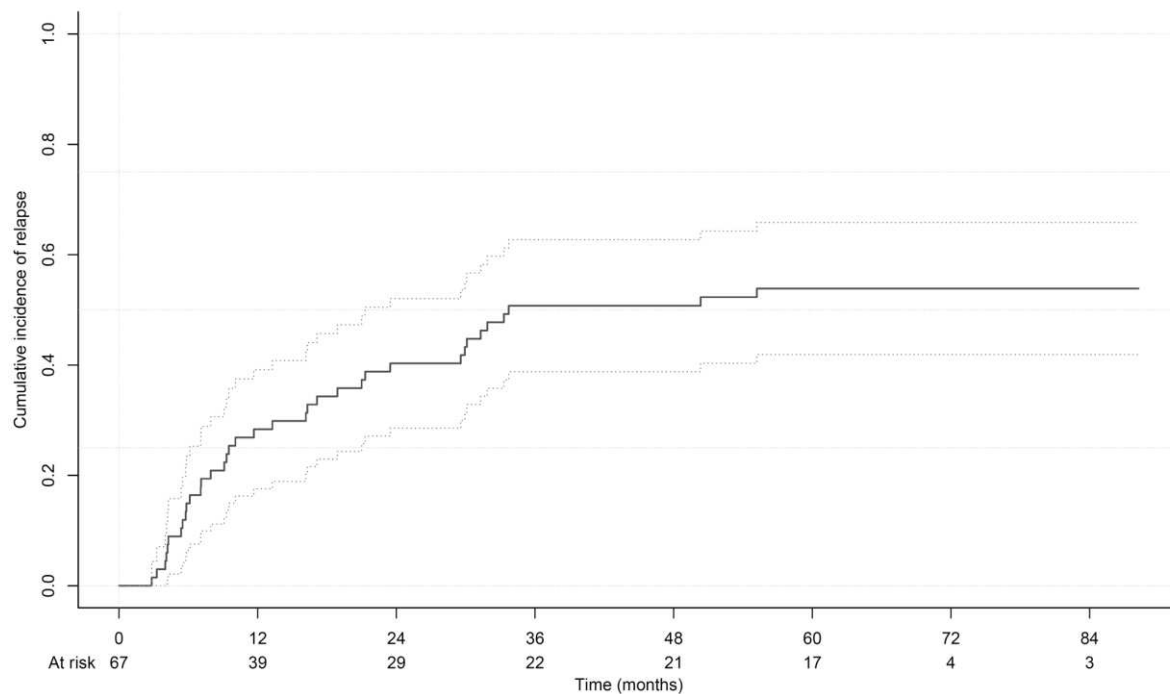
Figure 5. Follow-up of 10 patients positive for BCR-ABL<sup>T315I</sup> by ASO PCR at inclusion.



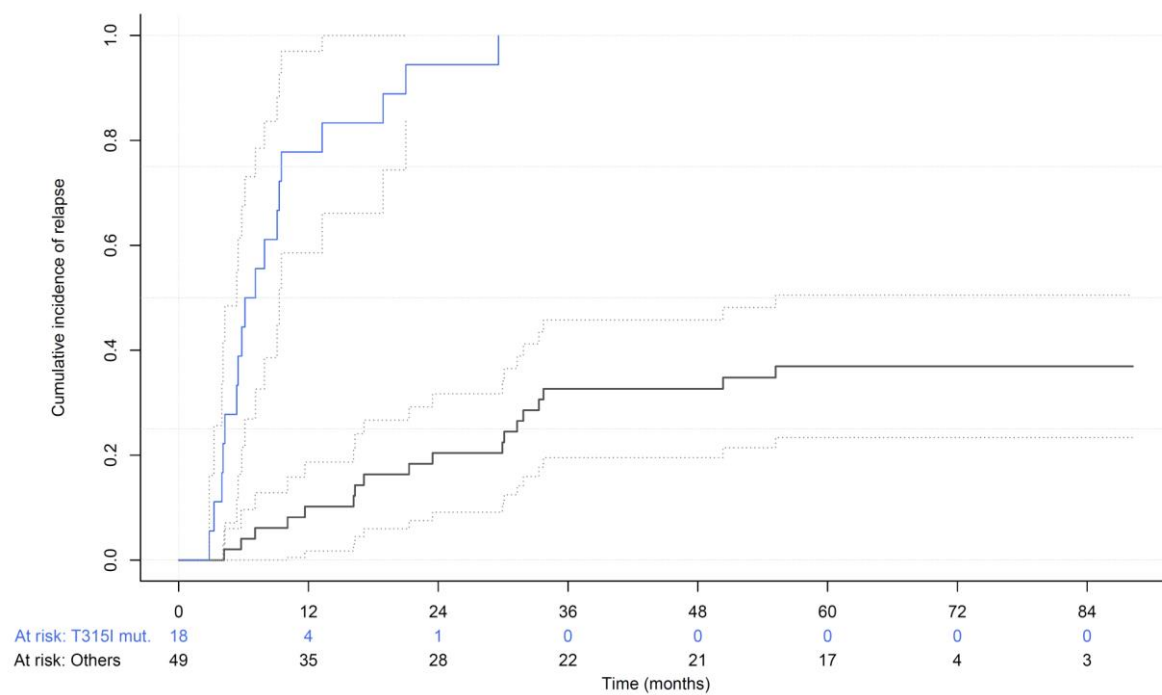
Allo HSCT, allogeneic hematopoietic stem cell transplantation; Post M, post maintenance; ● BCR-ABL<sup>T315I</sup> detected by ASO PCR at diagnosis; ○ BCR-ABL<sup>T315I</sup> not detected by ASO PCR during follow-up; ○ BCR-ABL<sup>T315I</sup> detected by ASO PCR during follow-up without hematologic relapse; ● BCR-ABL<sup>T315I</sup> detected by ASO PCR and Sanger sequencing at hematologic relapse.

**Figure 6. Cumulative incidence of relapse in all 67 patients in CR (6A) and in patients with BCR-ABL<sup>T315I</sup> > 0.05% as compared to other patients (6B) (Regression coefficient: 14.80 (95% IC, [7.03-31.2],  $p < 0.001$ ).**

6A



6B.





## Supplementary appendix

### Supplementary methods

For C944T(T315I) ASO RTQ-PCR, after extraction with TRIzol (Invitrogen) total RNA was titrated to 1 µg/µL with a NanoDrop 2000c UV-Vis spectrophotometer (ThermoScientific) and stored at -80°C. Complementary DNA (cDNA) was synthesized from 1 µg RNA with a reverse transcriptase M-MLV Kit® (Life Technologies) using Random Hexamer Primers (ThermoScientific). RTQ-PCR (BCR-ABL1, ABL1) was performed in 25 µl from one-tenth of the cDNA volume (100 ng RNA), using a thermocycler ABI7900HT (Life Technologies) with TaqMan reagent (Life Technologies) in standard mode (1 cycle of 2 minutes at 50°C then 10 minutes at 95°C followed by 50 cycles of 15 seconds at 95°C then 1 minute at 60°C). A highly sensitive allele-specific oligonucleotide real-time quantitative polymerase chain reaction (ASO RTQ-PCR) was used to quantify the BCR-ABL1<sup>T315I</sup> transcript ratio. The ASO (5'-GAGCCCCCGTTCTATATCATGAT-3') primer included one mismatch with the T315I allele (T) and two with the wild type allele (G and T). This ASO was used as forward primer, with reverse primer 5'-CGTTCACCTC CTGCCGG-3' and Taqman probe 5'-FAM-CCCTTCAGC GGCCAGTAGCATCTGA-TAMRA-3'. ASO RTQ-PCR reaction was performed in 25 µl from one-tenth of the cDNA volume (100 ng RNA) using the same reagent in a thermocycler, the only difference being an annealing temperature of 63°C. As the PCR efficiency was comparable between the two RTQ-PCR systems (BCR-ABL1<sup>T315I</sup> and ABL1), results are expressed as percentages of BCR-ABL1<sup>T315I</sup> and ABL1 copy number ratios (BCR-ABL1<sup>T315I</sup>/ABL1%), using the  $\Delta C_t$  method.

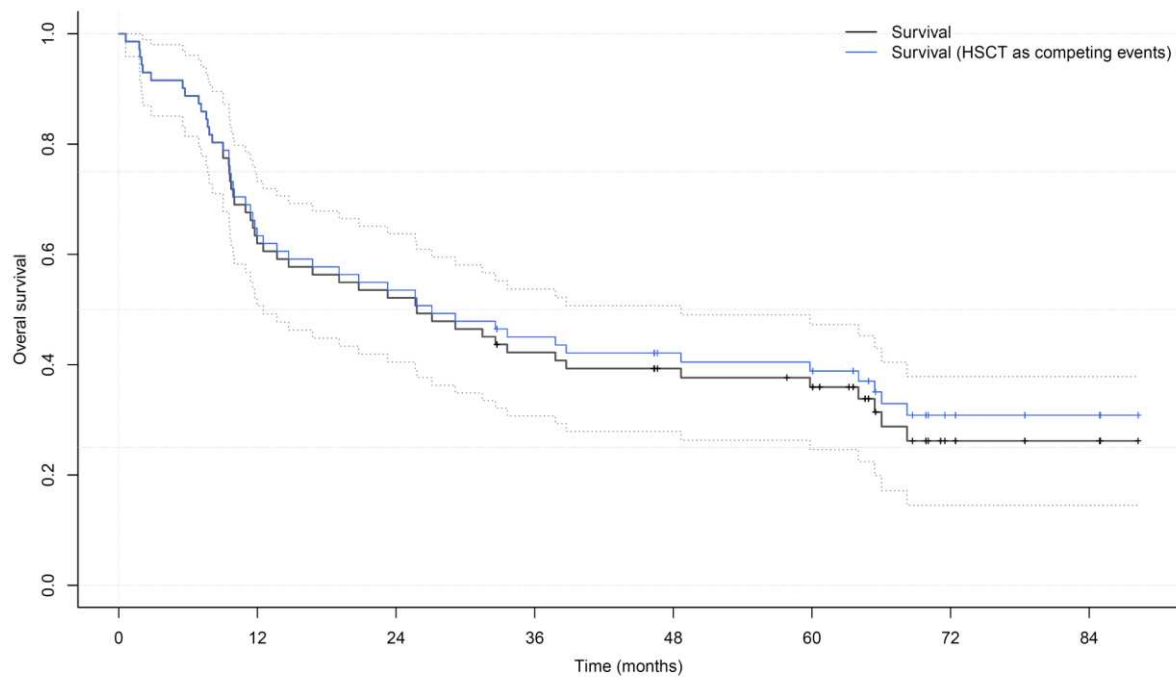
**Supplementary Table 2. Pleural effusions associated with dasatinib.**

Patient	Age, y	Sex	Dasatinib daily dose, mg/day	Latency, months	Associated symptoms	Grade	Pleural fluid
1	71	F	140	1.7	Pneumonia	3	Exudate
2	68	F	140	0.2	Pericarditis	2	ND
3	74	M	140	1.2	Pneumonia	3	ND
4	66	F	100	2.3	Pneumonia	2	ND
5	76	F	100	8.5	Bronchitis	2	ND
6	71	F	100	60.7	PAH	4	Exudate
7	65	M	100	4.2	None	3	Exudate

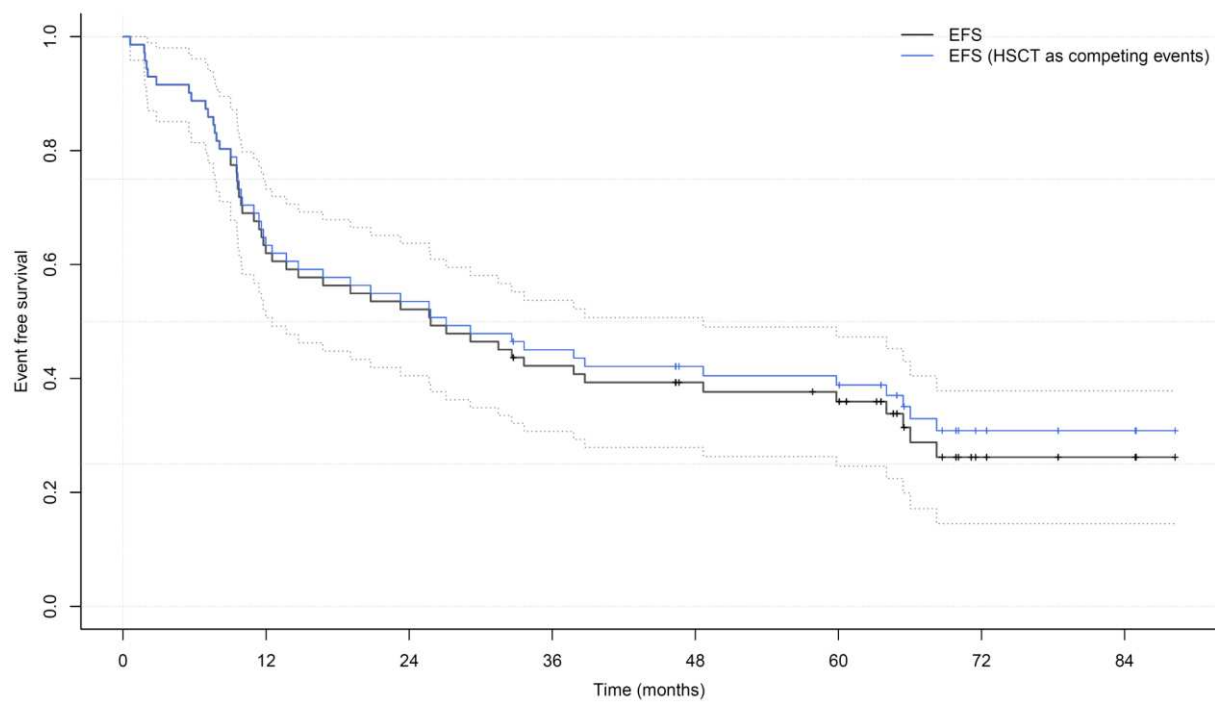
PAH, pulmonary arterial hypertension; M, male; F, female; ND, not done;

**Supplementary Figure 1.** Overall survival (OS, n=71)(S1A), event-free survival (EFS, n=71)(S1B) and relapse-free survival (RFS, n=67)(S1C) with and without censoring for allogeneic HSCT. HR for OS: 0.92 [95% CI, 0.61-1.37]; p=0.68; HR for EFS: 0.95 [95% CI, 0.64-1.39]; HR for RFS: 0.94 [95% CI, 0.63-1.42].

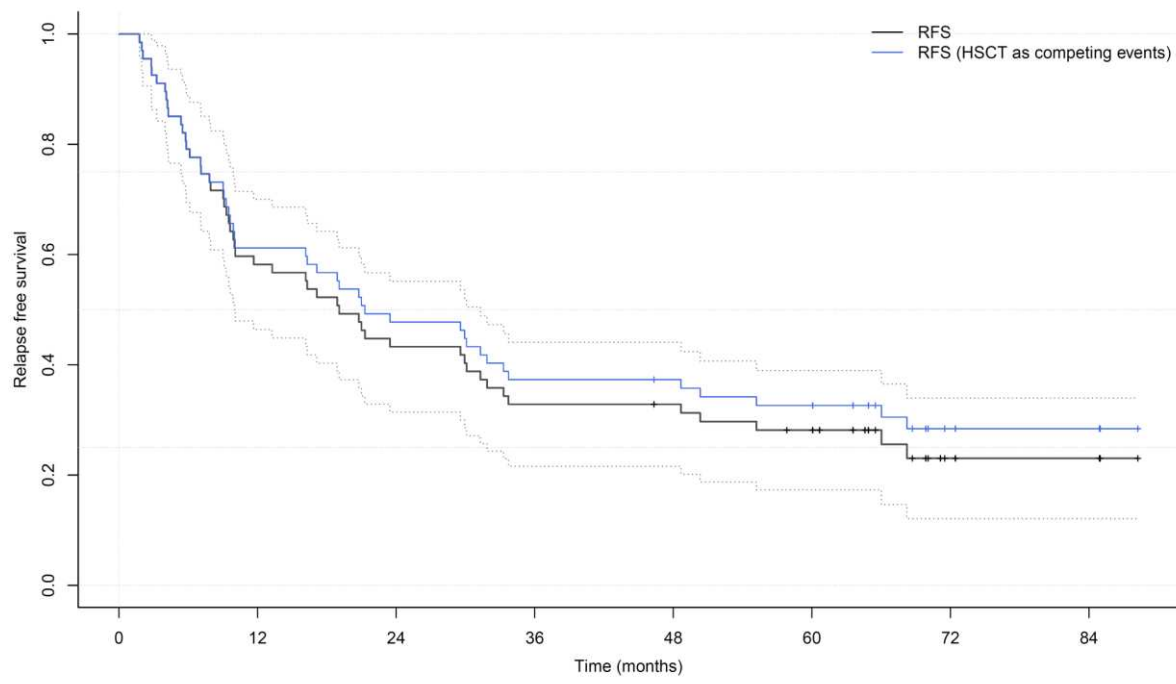
S1A



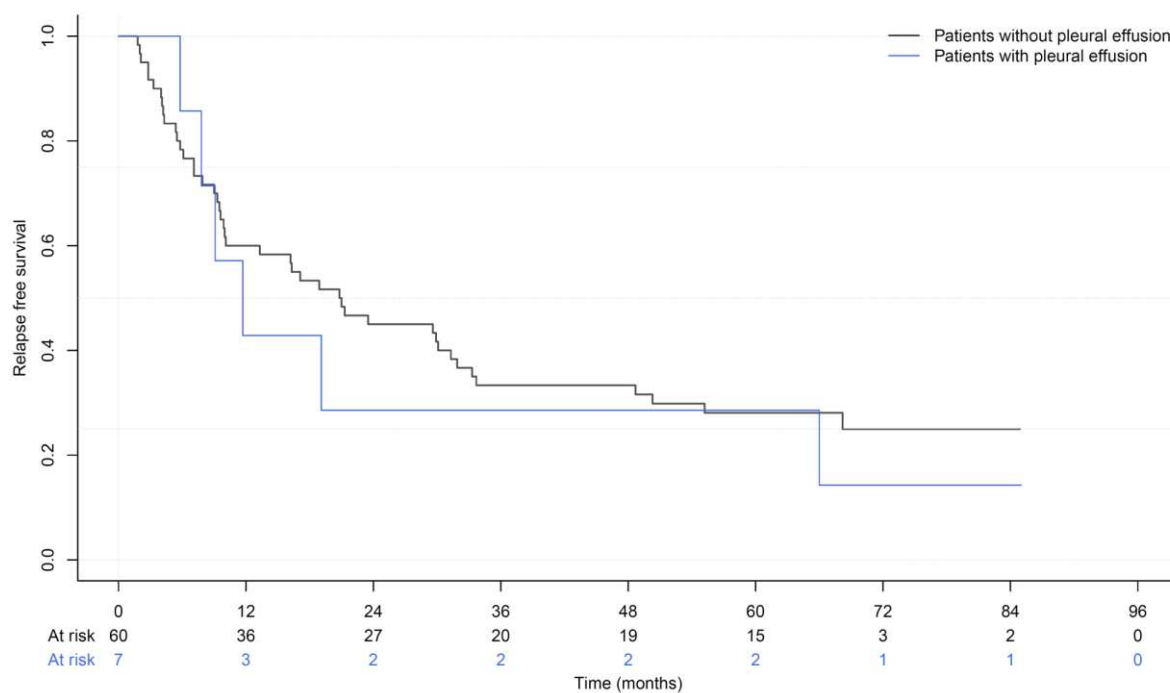
S1B



S1C



**Supplementary Figure 2:** Relapse-free survival in patients with (continuous line) or without (dashed line) pleural effusion (PE);  $p=0.6$  by the Log-rank (Mantel-Cox) test.



**Supplementary Figure 3:** Cumulative incidence of dasatinib discontinuation.

